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Determination of the myoglobin states in ground beef using non-invasive reflectance spectrometry and multivariate regression analysis

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ABSTRACT

Seventy-two samples of ground beef from *M. semimembranosus* of two 5 and two 1.5 year old animals were prepared. Two types of fat tissues from either beef or pork were added to the ground beef. The samples were prepared to contain predominantly deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) states on surfaces using selected methods based on chemical treatment (for MMb) and oxygen pressure packaging to induce the two other states. Reflectance spectra were measured on ground beef after three storage times. Partial least regression analysis was used to make calibration models of the desired myoglobin states. Validated models using leave-one-sample out cross validation gave, after correction and normalization, prediction errors of about 5%. Long term storage of ground beef was unsuitable for preparing pure MMb states due to gradual reduction of the pigment to DMb, presumably by bacteria.

1. Introduction

Color is an important quality attribute influencing the consumer's decisions to purchase the meat because they use color as an indicator of freshness. Discoloration that induces quality deterioration of meat, leads to a $1 billion annual revenue loss in the American meat industry (Mancini & Hunt, 2005; Smith, Belk, Sofos, Tatum, & Williams, 2000).

Myoglobin and hemoglobin are the pigments responsible for the color of meat, of which myoglobin is the main component in well bled muscle (Fernández-López, Sayas-Barberá, Pérez-Alvarez, & Aranda-Catalá, 2004). Myoglobin in meat exists predominantly in the three redox forms; bright red oxymyoglobin (OMb), purple deoxymyoglobin (DMb) and brownish metmyoglobin (MMb). CIE L*, a*, and b* values have been used to monitor changes in meat surface color over time (De Marchi, Penasa, Battagin, Pulici, & Cassandro, 2011; Fernández-López et al., 2004; Sheridan et al., 2007; Tapp, Yancey, & Apple, 2011).

There are different methods for calculating myoglobin states. By adding the chemicals sodium dithionite ($Na_2S_2O_4$) or potassium ferricyanide $K_3[Fe(CN)_6]$ it is possible to produce DMb and MMb, respectively (Wilson, Ginger, Schweigert, & Aunan, 1959). These commonly used methods are recommended by AMSA (1991) and were called Chemically Induced Myoglobin States (CIMS) (Khatri et al., 2012). Myoglobin forms can also be produced by modified atmosphere packaging (MAP) by adjusting the Oxygen Partial Pressure (OPP method) to approximately zero to produce DMb, to low O_2 to produce MMb, and to high O_2 to produce OMb (Sørheim, Westad, Larsen, & Alvseike, 2009; Taylor, Down, & Shaw, 1990). Fresh ground beef packaged in vacuum-bags without oxygen access, will consume all the oxygen present in the system in a few days, and will result in DMb as the primary pigment (Mancini & Hunt, 2005). MMb is formed at low oxygen pressure due to the fact that fast transformation to MMb occurs at 0.1–2% oxygen (Mancini & Hunt, 2005; Sørheim et al., 2009). To produce OMb, samples should be placed at low temperature (from 0 to 2 °C) in a high oxygen atmosphere, and flushed with 100% oxygen for 10 min, packaged in oxygen permeable film and scanned immediately (AMSA, 1991).

The high precision and accuracy in production of these three myoglobin states in solution will give good and simple equations for calculating mixtures of myoglobin states in new samples. Such predictive equations are based on isobestic points (Tang, Faustman, & Hoagland, 2004). For meat samples, however, light scattering needs to be handled in addition to preparing pure myoglobin states. Transmission spectra are often replaced by reflectance spectra for

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meat systems. The Kubelka–Munk transformation (Kubelka, 1948) to K / S values (K = absorbance coefficient; S = scattering coefficient) is frequently used to improve on linearity, reduce the contribution from scattering and make the spectra look more like transmission spectra. Recently different methods for scatter correction have emerged so that chemical effects can be more easily modeled. One such method called Extended Multiplicative Scattering (EMSC) (Martens & Stark, 1991), has been successfully used for elimination of uncontrollable path length or scattering effects, such as those caused by *e.g.* light scattering in reflectance spectroscopy (Khatri et al., 2012; Thennadil, Martens, & Kohler, 2006).

A simple approach to handle scatter has been to define the absorbance at 730 nm as zero. This is often used as an alternative to Krzywicki (1982) based on the variable log (1 / R), where R is reflectance, to calculate the myoglobin concentration of DMb, OMb and MMb using the isobestic wavelengths 474 nm, 525 nm, 572 nm and 610 nm. The same wavelengths are recommended by AMSA (1991). The existence of true isobestic points for scatter matrices such as meat should be questioned as neither the path of light nor myoglobin concentrations are known for each sample where myoglobin states are calculated.

Another mathematical approach other than recommending specific wavelength for all systems should be considered. The successful use of reflectance spectra in near infrared for fat, water and protein determination has relied on multivariate regression techniques (Næs, Baardseth, Helgesen, & Isaksson, 1996). Martens and Næs (1989) suggested that the method of Partial Least Square regression (PLS) is efficient in finding fundamental relations between two matrices. PLS makes it possible to model the multidimensional direction in the X matrices (*spectra*) that covary with the Y matrices (*myoglobin states*) (Wold, Sjöström, & Eriksson, 2001). This regression method was used by Khatri et al. (2012) to predict myoglobin states with lower prediction errors than possible if a few selected wavelengths were used. They also suggested that the PLS regression method seemed to partly compensate for lack of accuracy in preparing pure myoglobin states.

The main objective of this study was to extend the method proposed by Khatri et al. (2012) for predicting myoglobin states of whole meat to ground beef systems that have different scattering properties. The sub-objectives were: 1) to make a calibration model for predicting myoglobin states in stored (0–13 days) ground beef; 2) to evaluate the stability of the preparation method of pure myoglobin states on different stored minced samples; and 3) to compare regression models for intact muscles with those from ground beef.

2. Materials and methods

2.1. Raw material

Four days *post mortem* beef muscle (*M. semimembranosus*) with adhering fat tissue; from two 1.5 and two 5 year old animals were supplied by a local slaughter house (Fatland A/S, Oslo, Norway). In order to get variation two different fat tissues (beef and pork) and water were included. The type of breed was not identified. The muscles were transported and stored at 0–4 °C. The pork fat was supplied by HK Scan (Ruokatalo, Finland) and transported vacuum packaged and frozen to the lab.

2.2. Preparation of materials

2.2.1. Preparation of ground beef and fat

The beef muscle and pork and beef fat tissues were weighed and ground separately with a Seydelmann ME-130 (Seydelmann, Stuttgart, Germany) grinder, through a plate with 3 mm openings. The ground beef was prepared separately from each of 4 animals and mixed with additional fat from pork or beef in ratios of 86% w/w and 14% w/w respectively, and was manually mixed for 3 min. The mixture was then re-ground. To the ground beef and fat tissue (360 g) was added 40 g distilled water that was then manually stirred for about 2 min. The minces were varied because the predictive model for myoglobin states was to be used in another experiment where different solutions and fats were added.

2.2.2. Preparation method of myoglobin states

2.2.2.1. OMb. The ground mixtures were placed in boxes of amorphous polyethylene terephthalate (APET) trays, which were sealed with top film of ethylene vinyl alcohol (EVOH). The dimensions of the boxes were 20.5 × 14.8 × 3.5 cm. The trays and films (Wipak Multipet and Wipak Biaxer, both Wipak, Nastola, Finland) had oxygen transmission rates of 7 and 5 $cm^3/m^2/24$ h at 23 °C/50% relative humidity, respectively, at 50% relative humidity. A tray sealing machine (Promens 511VG, Kristiansand, Norway) was used for packaging of the OMb samples. One third of the OMb samples (8) were placed in trays filled with an atmosphere composed of 75% oxygen and 25% CO_2 (a premixture supplied by AGA, Oslo, Norway) after preparing the minces, as described in Section 2.2.1. The spectroscopic measurements were taken through the top film (EVOH) about 45 min after packaging in a high oxygen atmosphere and this time point was denoted as zero time. The rest of the samples (16) to be prepared later for the OMb state, were stored in EVOH bags (Biaxer) at 4 °C. One third of these samples (8) were removed on day 6, opened in order to be placed in trays with a high oxygen atmosphere, sealed and measured after 45 min. This procedure was repeated on the last 8 samples of ground beef on day 13.

2.2.2.2. MMb. The ground mixtures were oxidized with a 1% potassium ferricyanide $K_3[Fe(CN)_6]$ solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The ground mixtures were thoroughly soaked for 1 min. The excess $K_3[Fe(CN)_6]$ solution was drained off, and thereafter left covered with oxygen-permeable polyvinyl chloride film (PVC) at 4 °C. After 16 h the PVC film was removed and replaced with top film (EVOH). At this point the spectroscopic measurement was done through the top film and the time was denoted as time 0. This procedure was repeated on the stored ground beef at days 6 and 13.

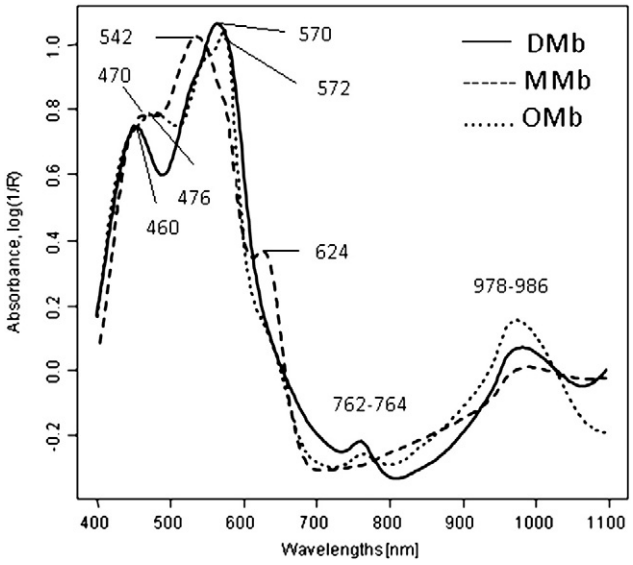

Fig. 1. Average EMSC (Extended Multiplicative signal Correction) spectra (from I = 64) *versus* wavelengths. "….." denotes OMb, "----" denotes MMb and "___" denotes DMb.